Spring 2015

Relating Personality and Body Size to Social Networks and Dispersal Behavior in Female Western Mosquitofish (Gambusia affinis)

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Relating Personality and Body Size to Social Networks and Dispersal Behavior in Female Western Mosquitofish (*Gambusia affinis*)

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1. INTRODUCTION

Humans are responsible for the breakdown of barriers between native flora and fauna around the globe. As a result, non-native species have the opportunity to expand their ranges well beyond their natural habitats, causing profound negative impacts to native species diversity (Vitousek et al. 1997). Studying the way in which invasive species spread and colonize new habitats an active field of study (Cote et al. 2010a). Physical characteristics (i.e. size, speed, etc.) as well as behavioral patterns of individuals (i.e. aggression, sociability, etc.) affect the colonization successfulness of an invasive species (Pintor et al. 2009). By understanding these factors, the spread of invasives can be predicted and even mitigated.

The western mosquitofish (*Gambusia affinis*) is native to the Mississippi River drainage and has naturally expanded its range eastward over time (Lowe 2000). Their invasion of over 40 countries is mainly due to introduction as a form of biological mosquito control. When introduced to new habitats, mosquitofish often eat the eggs of other organisms (fish, invertebrates, etc.). This can drive down the populations of economically important species and lessen biological diversity, despite having little effect on local mosquito populations (Lloyd 1986). *Gambusia* are also able to out-compete other species, as they are tolerant of a wide range of temperature, salinity, and various pollutants. For these reasons, the western mosquitofish is now listed by the Global Invasive Species Database as one of world's 100 worst invasive alien species (Lowe 2000). Additionally, a female mosquitofish can store sperm from a single mating for an entire breeding season and hatch up to six broods averaging 30-50 fry each (Lloyd 1986). Therefore, a single female in a new environment can be responsible for generating an entirely new colony. Investigations of the invasiveness of this species often focus on females for this reason.

In a previous study, western mosquitofish were found to be more likely to disperse downstream than upstream, and smaller individuals were more likely to disperse than their larger counterparts (Sargent et al., unpublished data). Cote et al. (2010a) found that asocial and bold individuals tend to disperse furthest. Additional studies showed that group composition plays a role in the dispersal of individuals, as individuals are more likely to disperse according to the average personality scores of the group. "Individuals from populations with more asocial

individuals or with more bold individuals are more likely to disperse regardless of their own personality type" (Cote 2010, group composition matters). Now, we seek to study the effects of size, environmental stressors (males and predators), and personality on shoaling and dispersal tendencies in the western mosquitofish.

In this study, personality is a loose term used interchangeably with behavioral syndrome/behavioral phenotype. These are repeatable differences in individuals' behaviors. Our work focused on sociability and boldness. Sociability has been defined as the tendency to shoal with other fish (Cote 2010a,b) and boldness is defined as the tendency to inspect a predator (Croft 2009) or to explore a novel environment. For our purposes, shoaling is the act of fish swimming together less than 15cm (~5 body lengths) from one another.

2. MATERIALS AND METHODS:

a. Study population

Fish were captured in early June 2014 at the University of Kentucky's Environmental Research Facility from several ponds and at Spindletop Research Farm from Cane Run using a combination of small mesh traps and seines. Fish were kept in groups of sixteen and marked with unique color tags to distinguish individuals from one another. Each fish was sedated using tricaine methanesulfonate (MS-222) and injected with a small amount of an acrylic paint and water mixture on either side of its dorsal fin. Using four colors, a total of 16 combinations were created. Fish were measured and weighed initially as well on the day of each test to monitor any major changes (e.g. dropping a brood).

b. Sociability (Cote)

To measure sociability, each fish was placed in a tank for 10 minutes and given the option to shoal with a stimulus group of fish. A tank measuring 30x25x50 cm was divided into three compartments: one large central compartment 25 cm long and two side compartments 12.5 cm long separated from the center by transparent barriers. A stimulus shoal composed of four females

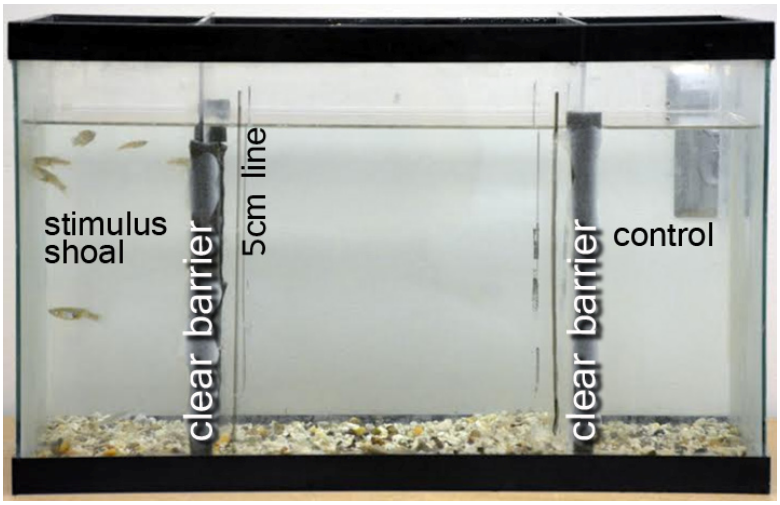

Fig. 1.1 Cote apparatus

and three males was held in one of the two side compartments with the opposing compartment left empty as a control. A focal fish was placed in the center compartment and allowed to acclimate for ten minutes. The fish was then observed for the following ten minutes and time spent shoaling within two centimeters of the shoal compartment was recorded. Sociability scores were measured as a proportion of time spent shoaling to total time in the tank (600 seconds) (adapted from Cote et al. 2010a).

c. Sociability and predator inspection (Croft)

Fish were given an additional opportunity to shoal with a stimulus group as well as an opportunity to inspect a predator to determine their sociability and boldness. A second tank measuring 30x30x75 cm was divided into three compartments separated from one another by opaque barriers. The first compartment (15 cm) held the focal fish for ten minutes while they acclimated to the tank. Centered in the middle compartment was a clear glass cylinder 7 cm in diameter filled with a stimulus shoal of four female mosquitofish. After a ten minute acclimation period, the first barrier was carefully lifted so as to avoid disturbing the focal fish. The focal fish was observed for ten minutes following its exit from the acclimation chamber. Time spent within five centimeters of the stimulus shoal was recorded. The second opaque barrier was then opened to reveal a large fishing lure made to resemble the fish's natural predators. Time spent "inspecting" the predator (passing within 5 cm of the predator) was recorded along with the number of inspections. Sociability scores were measured as proportions, similar to the Cote test. Boldness was determined by the number of predator inspections and time spent in proximity to the predator (adapted from Croft et al. 2009).

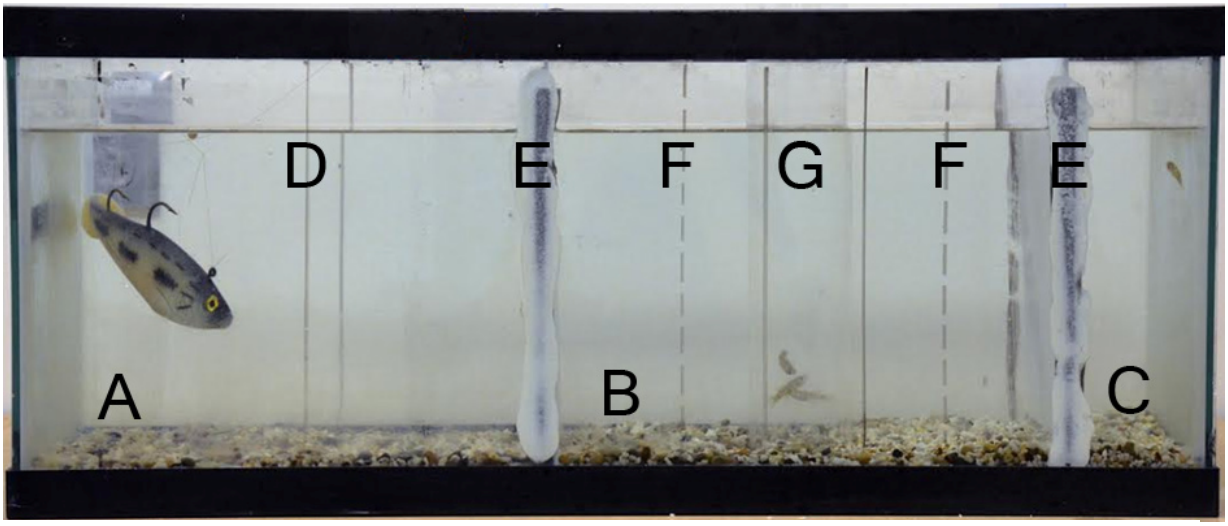

Fig. 1.2 Croft Apparatus. A. predator inspection chamber B. shoaling chamber C. acclimation chamber D. predator inspection threshold E. opaque barriers F. shoaling threshold G. clear glass tube with stimulus shoal

Every fish was run through each assay twice and scores were averaged in order to determine individual behavior phenotype (social/asocial, bold/timid).

d. Exploration

An exploration arena was constructed, measuring 100cm x 100cm with half flowerpots in two adjacent corners to serve as refuge. A focal fish was placed in a small, opaque container opposite the flower pots. After an acclimation period of ten minutes, a door was opened, allowing the fish to exit. Once free, the fish was remotely photographed from above once a second for five minutes. The photos were uploaded to ImageJ and analyzed using the manual particle tracking feature. The fish's trajectory was recorded and an area five centimeters to either side of the fish's path was measured. This resulted in a measured proportion of the tank that was "explored" by each fish as calculated by the area covered by the fish divided by the total area of the arena.

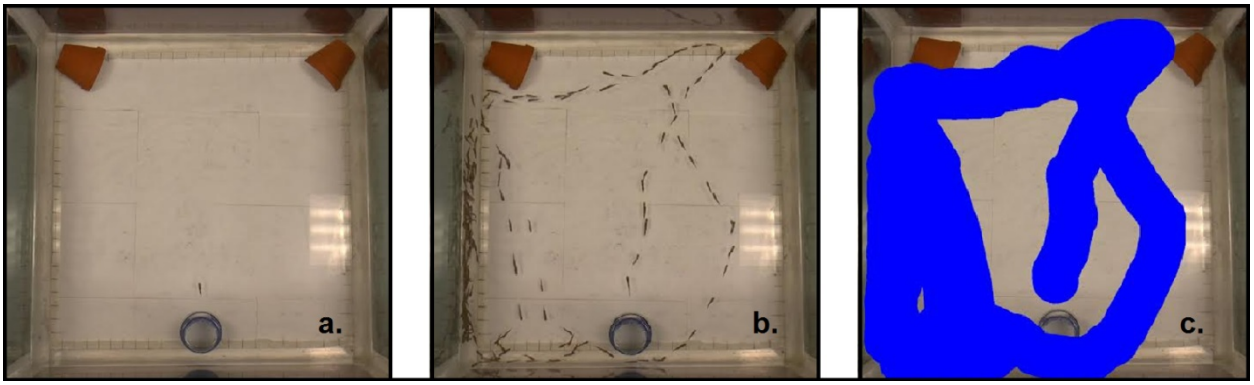

Fig 1.2 Example of the area explored by an individual. a. Fish is released from acclimation chamber. b. All 300 photos overlaid. c. Area explored

e. Social networking

After initial sociability and boldness scores were calculated for each of the fish in our study population, individuals were assigned to one of three focal groups, each arranged with a combination of large/small, social/asocial, and bold/timid individuals. To analyze social networking, groups were placed in a large seventy-gallon tank with one of three treatments: control, males present (a shoal of males equaling the number of females in the focal group), and predators present (two green sunfish housed in a clear jar in the center of the tank). After a ten-minute acclimation period, a pair of automated cameras took pictures of the tank once a minute for thirty minutes. These stills were analyzed for number and size of shoals, predator inspections, and to determine which fish tended to associate with one another most often. Fish were considered to be shoaling if they were observed within 15cm (five body lengths) of one

another. These interactions were used to build a social network matrix of each group. Boldness (proximity to the predators) was also noted.

f. Dispersal

In order to observe dispersal tendencies, an artificial stream was constructed within a 70-gallon tank, divided into three compartments connected to one another by variegated coverts cut in half to function as the "riffles" of a stream. A pump kept water continuously flowing in one direction to simulate a natural stream current. One focal group of females was placed in the center compartment at a time and given ten minutes to disperse. After ten minutes, the current was halted and fish were captured, noting which individuals stayed or dispersed "upstream" or "downstream." This was repeated a total of ten times for each of the three focal groups.

3. RESULTS

Sociability

Each individual was run through this test twice over the course of two weeks. Reactions of the fish to the stimulus shoal varied greatly, though the majority spent most of their time with their snouts pressed against the glass, attempting to swim toward the shoal behind the clear barrier. The largest females tended to spend the least time shoaling, while smaller individuals spent almost the entire 5 minutes of the test within the shoaling threshold. Due to all data being kept on University of Kentucky computers and lack of connection between the datasets, proper statistical software, and myself, results are currently in preparation by my mentor to be added as they are finalized.

Exploration

Individual females were run through this test once each and given 5 minutes to explore. A handful of outliers hid in corners or beneath the flowerpots set out as refuge. The rest, upon leaving the acclimation chamber, tended to swim continuously for most of the time period, some pacing enough times to cover almost the entire tank in blue after being tracked using ImageJ. As with sociability, the data remain at the University of Kentucky and are in need of further calculation to draw definite conclusions about behavior and its repeatability in relation to the previous measure of boldness: predator inspection.

Social networking

No difference in shoaling was found between control and predator trials, but a significant difference was found for treatment with males (Fig. 2.1), showing that shoal sizes are in fact

smaller when males are present. Figure 2.2 demonstrates examples of two social networks constructed by ICUNET. The first shows females in the control setting. The second shows interactions between females when males were present. Each node represents a specific females, with letters indicating their unique color identifier (RG = red to the left of the dorsal fin and green on the right, etc.). Connections between nodes represent individuals who were observed in a shoal together at least once (within 15cm of one another). It is clear that males disrupted the females' shoaling tendencies, breaking them apart into smaller groups, thus producing far fewer connections.

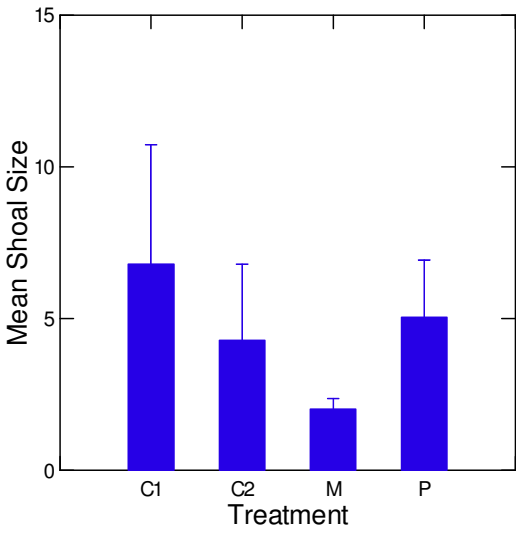

Fig. 2.1 Shoal sizes in relation to each experimental treatment: control, males, and predators

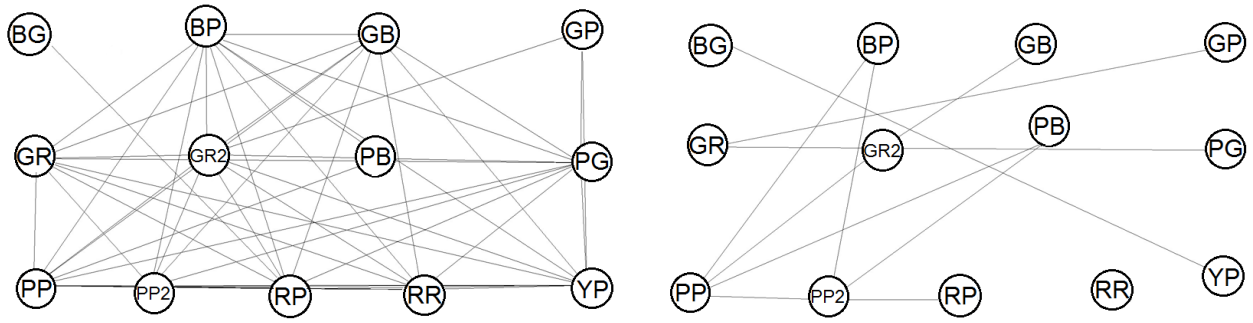

Fig. 2.2 Example of a social network structures for control and male treatments

Dispersal

Our results show a tendency for fish to disperse downstream most often (Fig. 2.3). Groups 2 and 3 show significant differences in the number of individuals that swam downstream rather than remaining in the center compartment. Very few fish, less than one per trial, swam upstream. We did not find any significant correlation between size and dispersal. It was equally likely that relatively large and small individuals disperse downstream. We found that behavioral phenotypes did not have a very strong correlation with downstream dispersal or non-dispersal (Fig. 2.4), although a significant proportion of upstream dispersers were asocial.

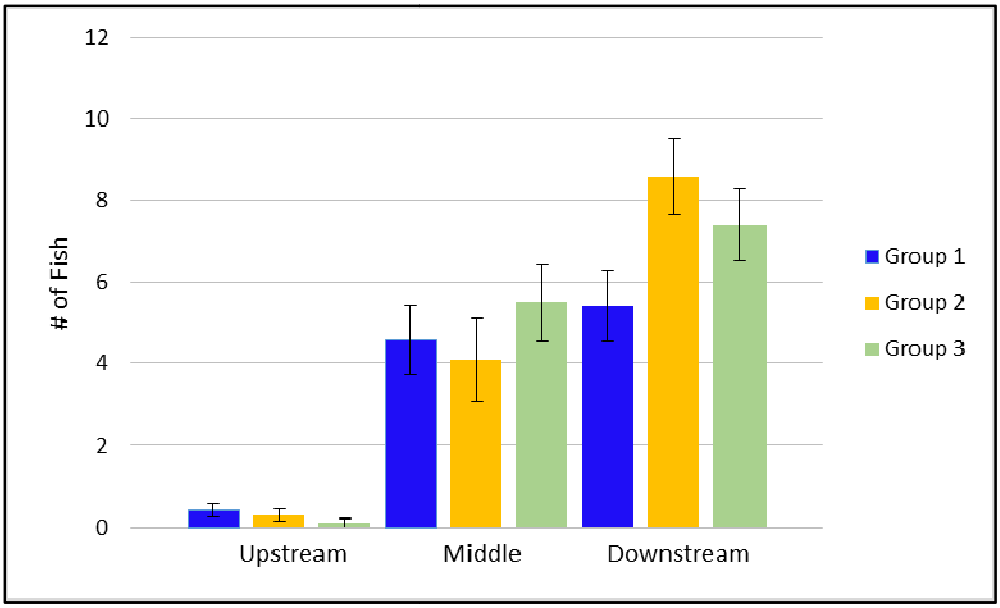

Fig. 2.3 Dispersal directions for 3 of our groups of 16 fish. Each group was placed in the stream a total of 10 times

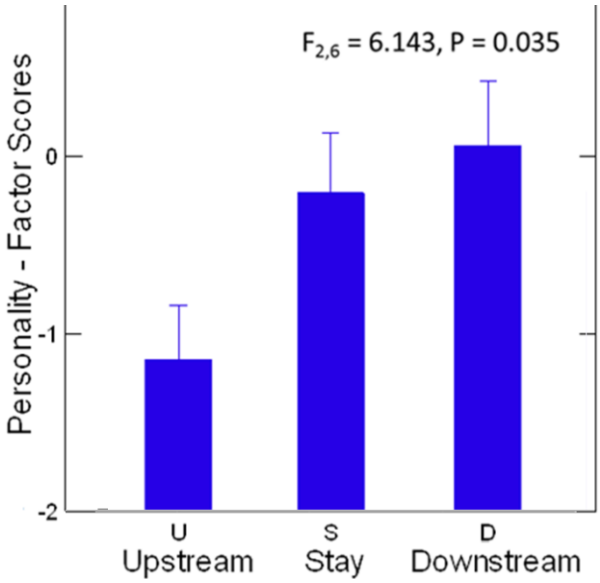

Fig. 2.4 Behavioral Scores and Dispersal. Lower factor scores indicate more asocial individuals with higher predator inspection rates

4. DISCUSSION

Sociability

It was noted through observation that larger females tended to spend less time shoaling than smaller individuals. As was found in a previous study (Sargent 2013, unpublished data) female

mosquitofish tend to show behavioral fluidity as a result of pregnancy. Those which were closer to dropping a brood tended to be less social, perhaps to distance themselves from other individuals to prevent cannibalism of their offspring. This was witnessed in our own tests, as one female gave birth during a trial and stayed far from the stimulus shoal while doing so. We expect to find a negative correlation between sociability and body size accordingly.

Social Networking

Ecological stressors do in fact affect shoal size and shoals were smallest when males were present. Males were allowed to swim alongside females, whereas predators were housed in a clear container within the tank. This may have skewed our results, as females may not have felt as though they were in any real danger with the predators in the tank.

Dispersal

Downstream dispersal was most common, appearing to be independent of body size and behavioral phenotype, with the exception of those that dispersed upstream being less social. Passive drift may be a confounding factor for dispersal. Being top feeders that tend to stay very close to the water's surface, the mosquitofish could have easily been carried away by the current, regardless of body size.

Future directions of this work involve seeking correlations between personality and condition-dependence (optimal behavioral decision depending on an individual's current condition, e.g. body size, reproductive state). Larger artificial streams that better mimic the natural environment may be used in coming trials. Finally, it may be helpful to determine the interactions between individual behavior and collective behavior with respect to the individual traits versus mean group traits.

This research was graciously funded by the National Science Foundation (NSF) and the Kentucky Science and Engineering Foundation (KSEF). Special thanks to the University of Kentucky's Suburban Ecology and Invasive Species Research Experience for Undergraduates program and especially to Dr. R. Craig Sargent for being a wonderful mentor.

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